

Poster Session II

melting curve analysis to classify positive samples into 2 groups, antifungal-resistant and antifungal-susceptible. **Conclusions:** Using a novel molecular beacon probe, we have developed a pan-*Candida* assay that can detect, quantify, and distinguish between antifungal-resistant and antifungal-susceptible *Candida* species. With further clinical validation, we hope that this assay will provide physicians a rapid means of identifying *Candida* infection directly from clinical samples and assist in institution of early therapy to reduce the hospital stay and high overall costs associated with management of candidemia.

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DEVELOPMENT OF A REAL-TIME PCR ASSAY TO DIAGNOSE INVASIVE ASPERGILLOSIS

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Background: Invasive *Aspergillus* causes significant morbidity and mortality in bone marrow transplantation recipients. In the absence of rapid, sensitive, specific, and quantitative diagnostic assays, it is estimated that > 90% of undiagnosed and untreated cases result in death. Here we report a rapid, sensitive, and specific real time PCR assay to diagnose *Aspergillus* infections. **Methods:** We set out to develop a real-time PCR method to detect and quantify 8 medically important *Aspergillus* species (*A. fumigatus*, *A. clavatus*, *A. flavus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. terreus*, and *A. versicolor*) in a single assay. We carefully aligned several *Aspergillus* multicopy rRNA genes to search for a target that is both specific for *Aspergillus* and conserved for all 8 species. To evaluate the diagnostic utility of a potential target, we performed real-time PCR on blood specimens that were spiked with serially diluted conidia from each of these 8 species. Additionally, we evaluated the effects of DNA extraction yield on the assay's sensitivity. For this purpose, we evaluated 3 commercially available DNA extraction kits: EZNA, Qiagen, and MagNA Pure DNA extraction methods. **Results:** Through sequence analysis, we designed a primer pair and TaqMan probe specific for a region of the 18S rRNA gene that is conserved for all 8 species. On application, our results demonstrate that this assay can detect and quantify as few as 100 copies of all 8 medically significant *Aspergillus* conidia per mL of spiked blood. In evaluating 3 commercially available DNA extraction kits, we found that the EZNA kit provided the maximum DNA yield and increased assay sensitivity. Moreover, we determined that mechanical disruption of the specimen by glass beads resulted in higher DNA yield and increased assay sensitivity. **Conclusion:** Developing a single molecular diagnostic assay for all medically important *Aspergillus* species is major challenge. Currently we are performing both retrospective and prospective studies to evaluate its diagnostic utility. Recently, the galactomannan-based assay showed promise in diagnosis of invasive aspergillosis; however, it still has high false-positivity and false-negativity. Furthermore, an optimum OD cutoff value has yet to be established for early diagnosis. Therefore, real-time PCR assay can provide an alternative means of diagnosis of invasive aspergillosis.

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RELATIONSHIP OF BK VIRAL LOAD IN BLOOD AND URINE WITH THE SEVERITY OF HEMORRHAGIC CYSTITIS (HC) AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Background: Hemorrhagic cystitis is a frequent and potentially serious complication after HSCT. An association between BK virus in the blood and/or urine and late-onset hemorrhagic cystitis has been described in literature. The role of BK virus in the pathogenesis of HC is currently unknown, but it has been suggested by the strong temporal correlation between the onset of viruria and HC. The goal of this study was to evaluate the relationship between BK virus load in blood and urine and the severity

of HC after HSCT. **Methods:** We retrospectively analyzed the records of 8 patients who had BK virus associated HC after HSCT seen at our institution between June 2002 and June 2004. All of the patients were male, with a median age of 45 years (range, 24–61 years). The severity of HC was graded according to the number of RBCs in the urine: grade 1 (RBCs <50), grade 2 (RBCs 50–100), grade 3 (RBCs > 100), or grade 4 (macroscopic hematuria with/without clots). Grade 1 and 2 were subdivided into grades 1a/2a (without symptoms) and 1b/2b (with symptoms of suprapubic pain and/or dysuria). BK viral load in blood and urine specimens was measured by a real-time PCR assay done by ViraCor Laboratories. The Shapiro-Wilks test was used to determine goodness of fit for normal distributions. The data were found to be nonnormally distributed, and so correlations were assessed using Spearman's test. **Results:** There was a significant correlation between BK viral load in the urine and the number of RBCs in the urine ($P = .0005$, 95% confidence interval [CI] = 0.1968–0.62550). There was also a significant correlation between BK viral load in the blood and BK viral load in the urine ($P = .0008$, 95% CI = 0.1807–0.6153). **Conclusion:** This study suggests a strong correlation between BK viruria and the severity of HC, as well as a strong correlation between BK viruria and BK viremia. More studies are needed to determine the implications of these correlations on early diagnosis and treatment of BK viral infection.

LYMPHOMA/MULTIPLE MYELOMA

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MODELED COST-EFFECTIVENESS ANALYSIS OF AUTOLOGOUS HAEMOPOIETIC STEM CELL TRANSPLANTATION COMPARED WITH STANDARD DOSE CHEMOTHERAPY FOR RELAPSED, AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Background: Salvage of patients with relapsed aggressive non-Hodgkin's lymphoma (NHL) before the mid-1990s involved standard dose chemotherapy (SDC). High-dose chemotherapy (HDC) combines the same initial drugs as SDC for 2–3 cycles, followed by high-dose regimes (conditioning) with autologous haemopoietic stem cell transplantation (AHST). In 1995, superior survival for HDC vs SDC was demonstrated in a European randomized trial (Philip et al. N Engl J Med 1995;333:1540–5), and HDC rapidly became the treatment of choice. Because HDC involves substituting conditioning treatment supported by AHST for 3–4 cycles of SDC, the cost of HDC is higher. The aim of the present study was to calculate the incremental cost-effectiveness ratio (ICER) for HDC compared with SDC using Australian costs and the European trial data. **Methods:** 21 patients transplanted from 1995 to 2002 with characteristics similar to those of the European trial were identified from the HDC database of the Newcastle Mater Hospital (NMH). All drug, transfusion, inpatient and outpatient attendance, and similar relevant data from start of salvage treatment up to 100 days after conditioning and AHST were obtained and costed. SDC costs required modeling, because all suitable patients are scheduled to receive HDC if possible, and thus no concurrent SDC arms exist. SDC cost for 6 cycles of combination chemotherapy was modeled from data available from the cycles received before HDC. The European trial survival data were used because NMH patients were treated only with HDC, patient numbers were small, and follow-up was short. A lifetime estimate of patient-years gained by HDC versus SDC was calculated from the area under survival curves (AUCs) of HDC and SDC from zero to infinity. Costs and benefits were discounted in advance at 5% per annum. The ICER was calculated according to the following formula: incremental cost ÷ incremental benefit = (costs_{HDC} – costs_{SDC}) ÷ (AUC_{HDC} – AUC_{SDC}). **Results:** Costs for HDC and